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Original article

A toxicity study of methanolic extract *of Calliandra surinamensis* seeds on liver functions in rodents

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Abstract: Medicinal plants and herbal drugs have widely been used in several diseases which contain highly active pharmacological agents. Several previous studies have mounted about the hepatotoxicity of these remedies which ranges from mild enzyme alterations to liver failure in humans and animals. This study aimed to evaluate the toxicity profile of the crude methanolic seed extract of *Calliandra surinamensis* on rat liver functions. An acute toxicity study was carried out using modified Lorke's method and sub-chronic toxicity was done following the Organisation for Economic Cooperative and Development guidelines with testing chemicals 423 and 407 to assess the effect of the seed extract on liver function alongside histopathology assessment of the liver. The haematological indices revealed no significant change in red blood cells and other haematological parameters. The average organ weight of the tested rats showed no significant differences. The histopathological examinations of the rat liver revealed no observable toxic injury to the tissue after treatment with the seed extract across all concentrations. There were desirable morphological vasoactive changes of dilatation and active congestion in the organ across all the concentrations which were dose-dependent. There was induced activation of the sinusoidal kupffer cells, which signified a boost to the local immune system of the liver. The nucleoli in the hepatocyte nuclei were remarkably conspicuous which implied an increase in the production of ribonucleic acid that is deployed in protein synthesis.

Introduction

The liver is a major metabolic organ of the body that produces protein and macromolecules and can break down a lot of toxic materials [1]. It cleans the blood by getting rid of the harmful chemicals produced by the body and excretes them towards the kidney for elimination [2]. The liver helps get rid of bilirubin, a waste product from the breakdown of red blood cells. A build-up of bilirubin in the body can lead to a condition known as jaundice [3]. The liver is a key player in the body's digestive system as everything taken into the body system passes through it. Therefore, the liver needs to stay healthy to do its job. It uses nutrients supplied to the body to make hundreds of substances needed. Thus, nuts, seeds,

legumes, and whole grains amongst others are very rich in healthy fats, antioxidants, vitamin E, and beneficial plant compounds [4]. Plant seeds are very rich in nutrients because they contain all the reserved nutrients such as proteins, essential amino acids, minerals, and vitamins that help in the growth and development of the plant. These reserved nutrients are what make cereals and legumes major food sources [5]. The account of this realization has opened up opportunities for the seeds, economically, and biologically. Legumes have been recognised for their role and significance in deficiency nutrient prevention and studies have shown that leguminous seeds contain numerous unassessed nutritional deposits in them [6]. *Calliandra surinam-ensis* is a leguminous plant, a tropical shrub widely known in the tropics for its ornamental and horticultural uses. The methanolic extract from leaves and bark fractions has demonstrated membrane stabilizing activity, and antimicrobial effects. Flavone glycosides have been detected in the stem bark, and their methanolic extract has also demonstrated antimicrobial activity [7]. The plant is a South American natively valued for its year-round floral display, and also recognised for its use in traditional medicine for the treatment of coughs, wounds, and other ailments [7]. The flower extract of *Calliandra surinamensis* has shown some significant levels of antioxidant activity [8]. The seed is a source of food, fat-soluble vitamins, and mineral nutrients [9 - 10], and the anticancer activity of the plant extract has been reported [11]. Therefore, this study aimed to examine the effects of the consumption of the seed on the liver functions of rats to determine its nutritional value and physiological effects.

Materials and methods

The *Calliandra surinamensis* pods containing the dry seeds were collected from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University Benin, Benin City, Nigeria and were authenticated by a Plant Taxonomist in the Department of Plant Biology and Biotechnology. The pods were cracked open to release the dry seeds which were pulverized into powder using a mechanical blender and extracted exhaustively using the Soxhlet apparatus with methanol as a solvent. The extract was concentrated by a rotary evaporator under reduced pressure and stored in the freezer until it is required for use.

Experimental animals: Twenty-four male Swiss mice, ranging from 26.9 to 31.0 g were used for the acute toxicity studies, and 16 male Albino Wistar rats weighing between 152 and 215 g were used for the sub-chronic toxicity. The mice were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy, University of Benin. Mice were allowed to acclimatize for two weeks before the commencement of the study. The approval of the Institutional Ethics Committee of the Faculty of Life Sciences of University of Benin was obtained with a reference number of LS19018.

Acute toxicity studies: **A**cute toxicity tests were conducted in mice following the modified Lorke's method [12]. 12 mice were grouped into four groups of three mice each using one group as control. Single dose levels of 10, 100, and 1000 mg/kg, respectively, of the methanolic crude extract were administered orally to the mice for the first phase. They were observed for 24 hours. In the second phase which was deduced from the first phase, 12 mice were grouped into four groups of three mice each. Three groups were treated with single doses of 1600, 2900, and 5000 mg/kg, respectively, of the seed extract orally while one group served as control. They were observed for 14 days. Acute toxicity tests revealed no deaths in mice after the oral treatment of the seed extract in the first and second phases.

Sub-chronic toxicity studies: Sixteen rats were selected by stratified randomization, and placed into four groups of four rats each. Three groups were treated with repeated doses of 200, 400, and 800 mg/kg of seed extract orally for 28 days, while one group served as control and was given normal food and distilled water. The weights of the animals for the 28 days were recorded. On day 29, the animals were sacrificed under chloroform anaesthesia, and

blood samples were drawn from the heart of each sacrificed animal. The samples were collected in a sterilized plain plastic test tube and allowed to stand for three hours to ensure complete clotting by the established guidelines [13]. The liver function enzyme activities alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) were determined by using the standard methods [14]. The total bilirubin and unconjugated bilirubin were determined using the published standard method [15] while the protein was determined as previously prescribed [16 - 17].

Histopathological analysis: This was carried out by fixing the dissected organ in 10.0% formal saline. The fixed tissues were completely dehydrated in ascending concentrations of alcohol (70%, 90%, 96%, and 100%). The tissues were placed in xylene to remove the alcohol, impregnated, and embedded with molten paraffin wax. They were allowed to solidify before sectioning into 4.0 µm using a microtome (Leica RM2235, UK), the 4 µm sections were placed on slides and stained with hematoxylin-eosin dye as described [18]. The stained slides were viewed using an optical photomicroscope (Olympus 230V 50/60 He, China), and camera (Eakins 12 Mega pixels at x40, x100, and x400 magnification). The haematoxylin reacts with the acidic components of the nucleus while eosin reacts with the basic components of the cytoplasm of cells and tissues.

Aspartate aminotransferase: AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine using 540 nm at 37 ^{o}C. Exactly, 0.5 µl of reagent 1, made up of phosphate buffer, L-aspartate and α-oxoglutarate were added to a test tube containing 0.1 ml of serum, mixed, and incubated for exactly 30 minutes at 37 ^{o}C. 0.5 ml of reagent 2, made up of 2, 4-dinitrophenylhydrazine was added, mixed, and allowed to stand for 20 minutes at 20 - 25 ^{o}C. Then 0.5 ml of sodium hydroxide solution was added, mixed and absorbance was read against the blank, also prepared, at 540 nm after five minutes. The AST concentration (U/L) was determined from the standard calibration table provided in the manual of Randox Lab. Ltd, UK Reagent Kit.

The Platelets Count determination: The assay was done by placing a small volume of diluted whole blood that was treated with a red cell lysing reagent, such as ammonium oxalate, in a counting chamber (hemocytometer), and counting platelets using phase-contrast light microscopy. The platelets count per µL of blood can be approximated by multiplying the number of platelets seen in one microscope field by 15,000.

The White Blood Cell Count: This was done by diluting blood with a Unopette system that contains a diluent (an agent glacial acetic acid) that lyses the red cells to remove them from view. A hemocytometer was charged with the diluted blood, and nuclei were counted in the appropriate areas of the grid using a light microscope.

Mortality and clinical signs: During the four week dosing period, all the animals were observed daily for likely clinical signs and mortality patterns once before dosing and rapidly after dosing.

Statistical analysis: The results were expressed as mean and the standard error of the mean (SEM). The data were further analysed using Latin Square Design. Three-way analysis of variance (ANOVA) with Turkey's post hoc test was used using the IBM SPSS version 20.0 for a test of significant difference at 95% level ($P < 0.05$) using a sample size of five.

Results

Liver function parameters: **Table 1** shows the activities of the three enzymes normally found in the liver, namely, alanine aminotransferase, (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT), respectively. The results for the ALT were 22.51 (200 mg/kg), 24.59 (400 mg/kg), and 29.53 (800 mg/kg), and 23.16 ± 0.06 (control). The values obtained for the AST were 47.98 (200 mg/kg), 45.86 (400 mg/kg), and 48.02 (800 mg/kg) and 47.22 ± 2.06 (control). The values obtained for the GGT were 0.46 (200 mg/kg), 0.65

(400 mg/kg), and 0.73 (800 mg/kg), and 0.49 (control). The values for bilirubin, 0.10 (200 mg/kg), 0.06 (400 mg/kg), 0.08 (800 mg/kg), and 0.07 (control). The values obtained for the total protein included 11.95 ± 0.33 (200 mg/kg), 15.29 ± 0.52 (400 mg/kg), 12.44 ± 0.94 (800 mg/kg) and 11.61 ± 0.45 (control).

Table 1: Effect of seed extract of *Calliandra surinamensis* on liver functions

Groups mg/kg	ALT (u/l)	AST (u/l)	GGT (u/l)	Total bilirubin (mmol/l)	Conjugated bilirubin (mmol/l)	Unconjugated bilirubin (mmol/l)	Total protein (mg/dl)	Albumin (mmol/l)
200	22.51±0.08	47.98±0.38	0.46±0.01	0.10±0.01	0.05±0.01	0.05±0.01	11.95±0.33	0.60±0.00
400	24.59±0.18	45.86±0.22	0.65±0.01	0.06±0.01	0.04±0.01	0.02±0.00	15.29±0.52	0.50±0.00
800	29.53±0.08	48.02±2.25	0.73±0.01	0.08±0.01	0.04±0.01	0.04±0.00	12.44±0.94	0.67±0.03
Control	23.16±0.06	47.22±2.06	0.49±0.01	0.07±0.02	0.03±0.01	0.04±0.01	11.61±0.45	0.63±0.03

ALT = alanine aminotransferase, AST = aspartate aminotransferase, GGT = gamma glutamyltranferase

Table 2 presents the results of percentage changes in weight of the rats with the dosage administered, and the results obtained at the end of the 28 days were as followings: 26.32% (200 mg/kg), 34.58% (400 mg/kg), and 33.7% (800 mg/kg), while the control was 28.4%. **Table 3** shows the results of the changes in the weight of the liver. The values did not show any significant change in the weight between the control, and the different concentration of the administered methanolic extract.

Table 2: Effect of seed extract of *Calliandria surinamensis* on the percentage change in the weight

Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28
200	0	05.76	17.65	22.03	26.32
400	0	14.84	23.20	29.97	34.58
800	0	07.80	18.62	27.63	33.67
Control	0	11.65	18.32	21.43	28.42

Table 3: Effect of the seed extract of *Calliandra surinamensis* on the average rat liver weight

Organs	**200 mg/kg**	**400 mg/kg**	**800 mg/kg**	**Control**
Liver (g)	0.035±0.000	0.037±0.004	0.034±0.000	0.034±0.002

Haematological indices: The values of the total white blood cells (TWBC) were 14.90 ± 1.93 (200 mg/kg), 14.30 ± 2.17 (400 mg/kg), 15.77 ± 0.50 (800 mg/kg), and 12.07 ± 1.87 (control). While the values of the red blood cells (RBC) were 6.90 ± 0.17 (200 mg/kg), 6.55 ± 0.25 (400 mg/kg), 7.23 ± 0.21 (800 mg/kg), and 7.14 ± 0.16 (control). The values obtained for haemoglobin (Hb) were 14.93 ± 0.34 (200 mg/kg), 14.33 ± 0.44 (400 mg/kg), 15.40 ± 0.59 (800 mg/kg), and 15.53 ± 0.34 (control). The values obtained for the blood platelets were 809.67 ± 59.01 (200 mg/kg), 963.33 ± 151.86 (400 mg/kg), 729.33 ± 42.55 (800 mg/kg), and 749.33 ± 34.21 (control).

Table 4: Effect of the seed extract of *Calliandra surinamensis* on haematological indices

Groups (mg/kg)	TWBC	RBC	Hb	Platelets
200	14.90±1.93	6.90±0.17	14.93±0.34	809.67±59.01
400	14.30±2.17	6.55±0.25	14.33±0.44	963.33±151.86
800	15.77±0.50	7.23±0.21	15.40±0.59	729.33±42.55
Control	12.07±1.87	7.14±0.16	15.53±0.34	749.33±34.21

TWBC = Total white blood cells, RBC = Red blood cells, Hb = Haemoglobin

Histopathological changes in the liver with seed extract of calliandra surinamensis: Both the control (Plate 1, H & E x 400) and 200 mg/kg (Plate 2) body weight showed normal hepatocytes, and nucleoli progressively became more conspicuous with the liver pointing at an increase in protein synthesis, and active vascular congestion. At 400 mg/kg body weight (Plate 3), there was mobilisation of sinusoidal kupffer cells, signifying activation of the local immune system of the liver. While at 800 mg/kg body weight (Plate 4), the nucleoli were conspicuous at this concentration, and the kupffer cells became floridly activated and looked like giant cells.

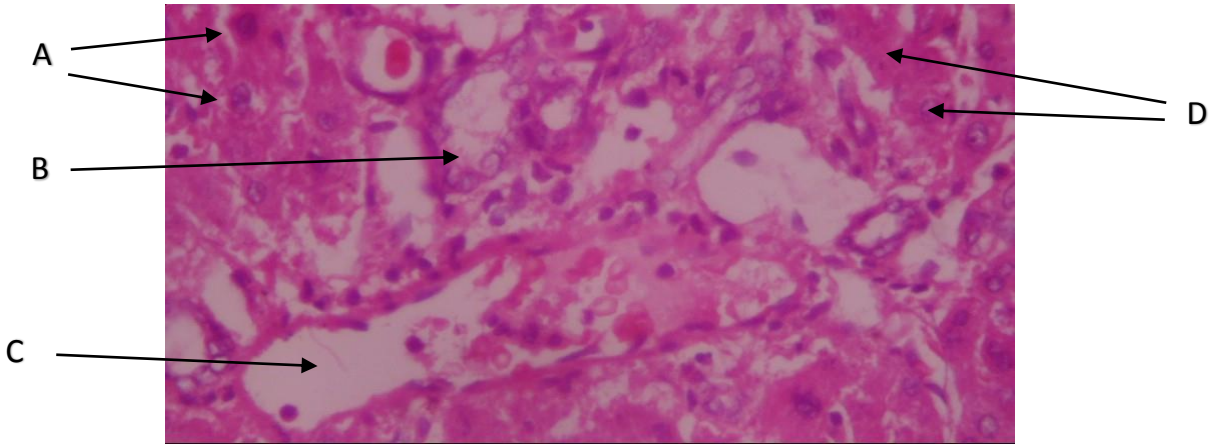

Plate 1: A: control composed, B: hepatocytes, C: bile duct, D: portal vein and sinusoids

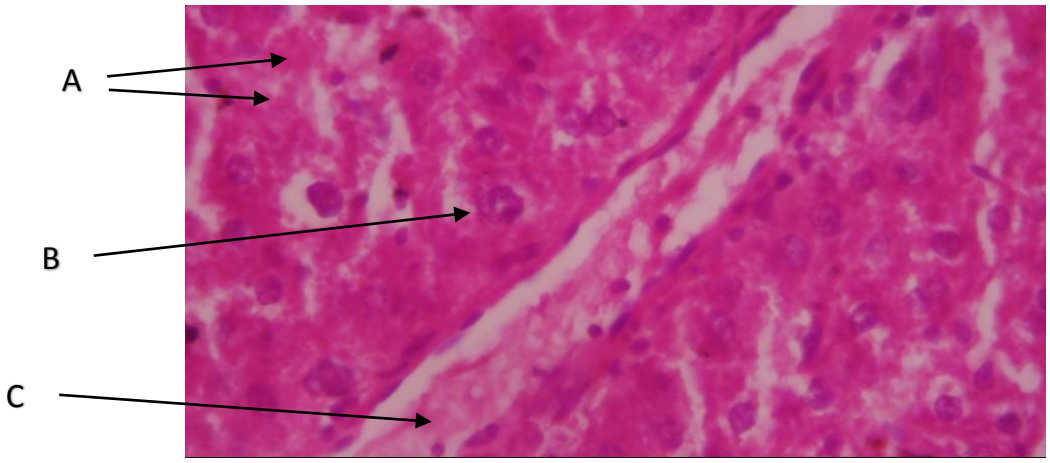

Plate 2: Liver of rat given 200 mg/kg C. surinamensis showing
A: normal, B: hepatocytes, C: nuclei containing conspicuous nucleoli and active vascular congestion

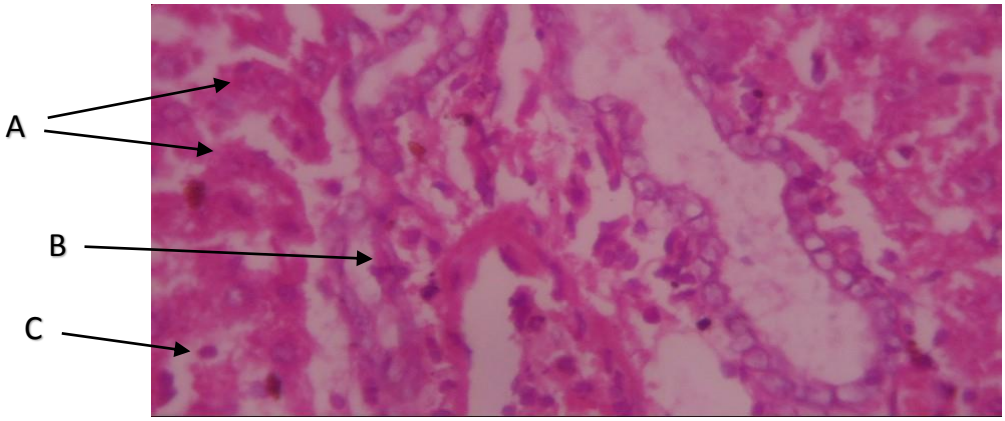

Plate 3: Liver of rat given 400 mg/kg C. surenamensis showing
A: normal hepatocytes, B: bile ducts, C: kupffer cell activation

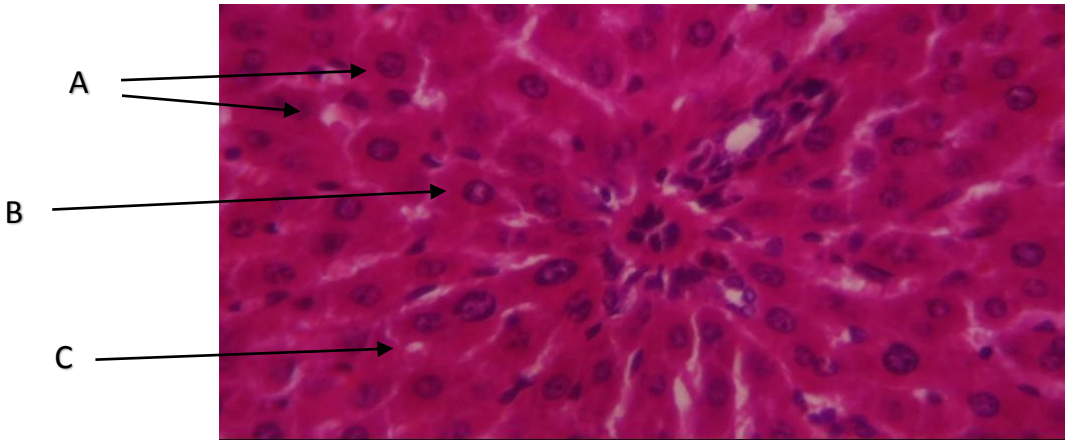

Plate 4: Liver of rat given 800 mg/kg C. surinamensis showing
A: normal hepatocytes, B: nuclei containing conspicuous nucleoli, C: kupffer cell activation

Discussion

In this study, the three liver enzyme activities (ALT, AST, and GGT) at the different doses were found not statistically different when compared with the values for the control. ALT is an enzyme found mostly in the liver that helps in the breakdown of proteins in the body. It is a biomarker for liver health [19]. AST is an enzyme found in the liver that helps in amino acid metabolism [20]. GGT is an enzyme mostly found in the liver which is commonly used as a diagnostic maker for liver diseases and functions critically in the degradation and synthesis of glutathione [21]. The activities obtained for total, conjugated and unconjugated bilirubin, for the different doses compared to the control were insignificant. Thus, bilirubin is a metabolic waste produced from the breakdown of red blood cells. Elevated levels of bilirubin are an indication of malfunctioning of the liver [22 - 24]. The findings obtained for the total protein and albumin for the different doses of the crude extract compared to the control were slightly higher although it is statistically insignificant. The total protein is also known as serum protein and is made up of albumin and globulin which plays a complementary role in helping to maintain healthy body functions and boost immunity, and also a measure for growth [25]. Except for 200 mg/kg dosage of the crude extract, there was a significant increase in the body weight of the rats compared to the control. This implied that the crude extract contributed to the growth of the rats and assisted in vital metabolic processes [26 - 27]. There was no significant change in the weight of the liver

at the different dosages compared to the control which showed that there was no inflammation of the organ [28]. There was a slight increase in the TWBC values for all the dosages of the crude extract compared to the control which indicated a boost in the immunity level of the rat [29]. There was no significant difference in the values of the RBC obtained with the crude extract and the control. A reduction in the values would have resulted in anaemic conditions [30]. In addition, there were no significant differences in the values obtained for the haemoglobin and platelet between the administered dosage and the control. The functions of the haemoglobin and platelet have previously been described [31, 32]. The findings of liver histopathological analyses revealed normal hepatocytes, and nucleoli progressively became more conspicuous which showed an increase in protein synthesis, active vascular congestion, mobilisation of sinusoidal kupffer cells which was dose-dependent, signifying activation and boost of the local immune system of the rat liver (Plates 2, 3, 4) [33 - 35].

Conclusion: In the acute toxicity of *Calliandra surinamenses* seed extract administered orally to rodents, there was no mortality and in sub-chronic toxicity, there were no behavioural or observable toxic effects on the rat liver. There was an increase in the weight of the rats indicating growth with no impairment in the liver functions but rather a marked improvement and activation of the sinusoidal kupffer cells which is an indication of enhanced immunity. Thus, it is concluded that the methanolic crude extract of *Calliandra surinamenses* is safe for the rat liver.

Author contribution: EEII conceived, and designed the study, PNE collected data and performed the analysis. IGE contributed to data analysis, data interpretation and histopathological analysis. UO contributed to collecting data and data analysis. MII contributed in histopathological and biochemical analysis. All the authors drafted, revised the manuscript and approved the final version of the manuscript and agreed to be accountable for its contents.
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Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission have completely been observed by authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

References

1. Nguyen-Lefebvre AT, Horuzsko A (2015) Kupffer cell metabolism and function. Journal of Enzymology and Metabolism. 1 (1): 101. PMCID: PMC4771376.
2. Haussinger D (2011) Liver regeneration. De Gruyter. 214. doi.org/10.1515/9783110250794. ISBN: 9783110250794.
3. Van Wagner LB (2015) Evaluating elevated bilirubin levels in asymptomatic adults. Journal of the American Medical Association. 313 (5): 516-517. doi: 10. 1001/jama. 2014.12835.
4. Smith SH (2017**)** Using albumin and preabumin to assess nutritional status. Nursing. 47 (4): 65-66. doi: 10.1097/01.NURSE.0000511805.83334.df.
5. Sabella PA, Larkins BA (2009) The development of endosperm in grasses. Plant Physiology. 149 (1): 14-26. doi: 10.1104/pp.108.129437.
6. Audu SS, Aremu MO (2011) Nutritional composition of raw and processed pinto bean (Phaseolus vulgaris L) grown in Nigeria. Journal of Food Agriculture and Environment. 9 (3): 72-80. Corpus ID: 137755699.

7. Irabor EEI, Falodun A, Obasuyi O, Ofoegbu CO, Abiodun SO, Umujeyan K (2007) Antimicrobial evaluation of methanolic extract of *Colliandra surinanamenses* on some pathogenic organisms. Acta Poloniae Pharmaceutica. 64 (5): 449-451. PMID: 18540166.
8. Falodun A, Irabor EE (2008) Phytochemical, proximate, antioxidant and free radical scavenging evaluations of *Calliandra surenamensis*. Acta Poloniae Pharmaceutica. 65 (5): 571-575. PMID: 19051604.
9. Irabor EEI, Onukwugha PU, Eze PN, Ogbeide OK (2020) Extraction and characterization of fat soluble vitamins from oil of *Calliandra surinamensis* seed. Journal of Chemical Society of Nigeria. 45 (3): 520-525.
10. Irabor EEI, Eze PN, Unuigbe CA, Imafidon MI, Omoruyi U, Emmanuel V (2023) Proximate analysis and mineral determination of *Calliandra surinamenses* seed. Journal of Chemical Society of Nigeria. 48 (1): 183-191. doi.org/10.46602/jcsn.v48i1.859.
11. Falodun A, Uzoekwe AS, Shengxiang Q (2010) Phytochemical, anticancer and antioxidant evaluation of potential chemical constituents of Calliandra surinamensis. Nigerian Journal of Biotechnology. 21: 55-59.
12. OECD/OCDE (2001) OECD guideline for testing of chemicals. Test guidelines 423: Acute oral toxicity–acute toxic class method. Office of Economic and Community Development, Paris, France. 423: 1-14.
13. OECD (2008) OECD guidelines for testing of chemicals. Test guidelines number 407: Repeated dose 28-day oral toxicity in rodents. Office of Economic and Community Development, Paris, France.
14. Magili ST, Beatanglang IB (2018) Toxicity study of aqueous leaves extract of *Jatropha gassypiifolia* from Nigeria in albino rats: serum biochemistry and histopathogical evaluation. International Journal of Biochemistry Research and Review. 21 (3): 1-12. doi: 10.9734/IJBCRR /2018/41307.
15. Doumas BT, Perry BW, Sasse EA, Straumfjord JV (1973) Standardization in bilirubin assays: evaluation of selected methods and stability of bilirubin solutions. Clinical Chemistry. 19 (9): 984-993. PMID: 4744825.
16. Lowry OII, Rosebrough NJ, Farr AI, Randall RJ (1951) Protein measurement with the folin-phenol reagent. The Journal of Biological Chemistry. 193 (1): 265-275. PMID: 14907713.
17. Ojeaburu SI, Eriyamremu, Otokiti IO, Uanseoje SO (2010) Stress enzymes and atpases in gastrointestinal tissues of rabbits fed boiled whole unripe plantain (Musa sapientum) extract and unripe pawpaw (Carica papaya). Journal of Science Engineering and Technology. 17 (2): 9441-9455.
18. Drury RAB, Wallington EA (2013) Carletons Histological Technique. 16th ed., Oxford University Press, London, UK. 124-136. ISBN: 978-0192613103.
19. Hirotsu K, Goto M, Okamoto A, Miyahara I (2005) Dual substrate recognition of aminotransferase. Chemical Record. 5 (3): 160-172. doi: 10.1002/tcr.20042.
20. West MB, Wickham S, Leslie M, Ryan E, Li C, Hanigan MH (2011) Autocatalytic cleavage of human gamma-glutamyi transpeptidase is highly dependent on N-glycosylation at asparagine 95. The Journal of Biological Chemistry. 286 (33): 28876-28888. doi: 10.1074/jbc.M111. 248823.
21. Smith ME, Morton DG (2010) Liver and biliary system. The digestive system. 3rd Ed. Elsevier. 85-105. ISBN: 9780702083761.
22. Vasavda C, Kothari R, Malla AP, Tokhunts R, Lin A, Ji M, Ricco C, Xu R, Saavedra HG, Sbodio JI, Snowman AM, Albacarys L, Hester L, Sedlak TW, Paul BD, Snyder SH (2019**)** Bilirubin links heme metabolism to neuroprotection by scavenging superoxide. Cell Chemical Biology. 26 (10): 1450-1460. doi: 10.1016/j.chembiol.2019.07.006.
23. Amin SB, Karp JM, Benzley LP (2010) Unconjugated hyperbilirubinemia and early childhood caries in a diverse group of neonates. American Journal of Perinatology. 27 (5): 393-397. doi: 10.1055/s-0029-1243314.
24. Abdullahi SM, Yakubu AM, Bugaje MA, Akuyam SM (2018) Serum total protein and albumin levels among malnourished children. Nigerian Journal of Paediatrics. 45 (1): 15-18. doi: 10.4314/njp.v45i1.4.
25. Owens FN, Gills DR, Secrist DS, Coleman SW (1995) Review of some aspects of growth and development of feedlot cattle. Journal of Animal Science. 73 (10): 3152-3172. doi: 10.2527/ 1955.73103152x.
26. Afolayan RA, Adeyinka IA, Lakpini CAM (2006) The estimation of live weight from body measurements in Yankasa Sheep. Czech Journal of Animal Science. 51 (8): 343-348. doi: 10.17221/3948-CJAS.
27. Petterino C, Argentino-Storino A (2006) Clinical chemistry and haematology historical data in controlled Sparague-Dawely rats from preclinical toxicity studies. Experimental and Toxicological Pathology. 57 (3): 213-219. doi: 10.1016/j.etp.2005.10.002.
28. Koyama Y, Brenner DA (2017) Liver inflammation and fibrosis. The Journal of Clinical Investigation. 127 (1): 56-64. doi: 10.1172/JCI88881.
29. Alberts B, JohnsonA, Lewis J, Raff M, Roberts K, Walter P (2002) Molecular biology of the cell. Annals of Botany. 91 (3): 401. doi: 10.1093/aob/mcg023.

30. Al-Naseem A, Sallam A, Choudhury S, Thachil J (2021) Iron deficiency without anaemia: a diagnosis that matters. Clinical Medicine. 21 (2): 107-113. doi: 10.7861/clinmed.2020-0582.
31. Giardina B, Messna I, Scatena R, Castagnoda M (2008) The multiple functions of hemoglobin. Critical Reviews in Biochemistry and Molecular Biology. 30 (3): 165-196. doi: 10.3109/ 10409239509085142.
32. Thomas C, Lumb AB (2012) Physiology of haemoglobin. Continuing Education in Anaesthesia, Critical Care & Pain. 12 (5): 251-256. doi.org/10.1093/bjaceaccp/mks025.
33. Tomioka M, Iinuma V, Okinaga K (2000) Impaired kupffer cell function and effect of immunotherapy in obstructive jaundice. The Journal of Surgical Research. 92 (2): 276-282. doi: 10.1006/jsre.2000.5868.
34. Kolios G, Valatas V, Kouroumalis E (2006) Role of kupffer cells in pathogenesis of liver disease. World Journal of Gastroenterology. 12 (46): 7413-7420. doi:10.3748/wjg. v12.i46.7413.
35. Zhang L, Bansal MB (2020) Role of kupffer cells in driving hepatic inflammation and fibrosis in HIV infection. Frontiers in Immunology. Frontiers in Immunology. 11: 1086. doi: 10.3389/fimmu.2020.01086.